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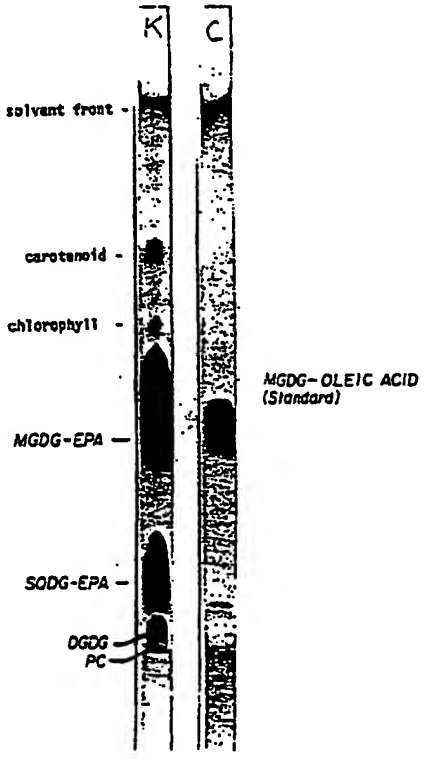
(57) Abstract

There is disclosed an anti-inflammatory composition containing eicosapentaenoic acid (EPA) bearing monogalactosyldiacylglycerol (MGDG-EPA) at high levels. Additional components of the composition include digalactosyldiacylglycerol (DGDG), phosphatidylcholine (PC), chlorophylls and carotenoids. Methods for making the anti-inflammatory composition by extraction of marine algae, as well as methods of treating inflammation by administering an effective amount of the composition to an animal, are also disclosed.



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Description

ANTI-INFLAMMATORY COMPOSITIONS CONTAINING EICOSAPENTAENOIC ACID BEARING MONOGALACTOSYL-DIACYLGLYCEROL AND METHODS RELATING THERETO

Technical Field

This invention relates generally to an anti-inflammatory composition and, more specifically, to an anti-inflammatory composition derived from marine algae having a high content of eicosapentaenoic acid bearing monogalactosyldiacylglycerol (MGDG-EPA).

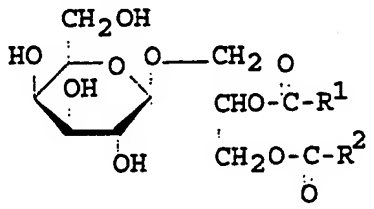
Background of the Invention

Monogalactosyldiacylglycerides (MGDG) may generally be obtained from a number of higher plant sources (including vegetables such as lettuce, broccoli, wheat, and alfalfa), from the central nervous system of animals, and from a variety of macro- and micro-marine algae. However, monogalactosyldiacylglycerides containing the polyunsaturated fatty acid eicosapentaenoic acid ("EPA") are only found in marine algae. More specifically, monogalactosyldiacylglycerides with the highest content of eicosapentaenoic acid are found in cold water marine micro-algae species. These eicosapentaenoic acid bearing glycerides (i.e., MGDG-EPA) are formed along with many other algal products, thus making the purification and isolation of useful quantities of these materials complex and burdensome.

Accordingly, there is a need in the art for a process for making compositions containing high concentrations of MGDG-EPA. In addition, there is a need in the art for methods relating to the use thereof. The present invention fulfills these needs, and provides further related advantages.

Summary of the Invention

Briefly stated, in one embodiment of the present invention, an anti-inflammatory composition comprising a high content of MGDG-EPA is disclosed. As used herein, "MGDG-EPA" is a monogalactosyldiacylglyceride wherein at least one of the acyl moieties is eicosapentaenoic acid esterified to the glycerol backbone, as represented by the following structure I:

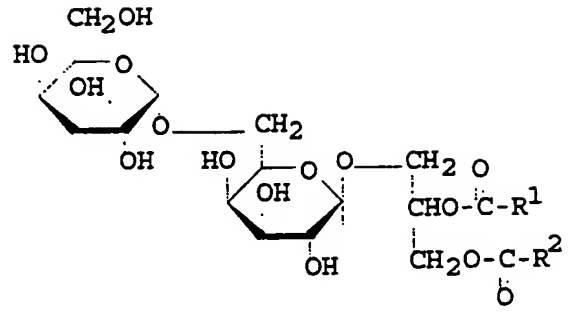


I

1,2-diacyl-(β-D-galactopyranosyl(1'→3))-*sn*-glycerol

wherein R^1 and R^2 represent a hydrocarbon chain of a fatty acid containing from 10 to 25 carbon atoms and from 0 to 6 carbon-carbon double bonds, and wherein at least R^1 or R^2 is the hydrocarbon chain of eicosapentaenoic acid. MGDG-EPA is present in the composition in an amount ranging from 35 to 95 percent by weight of the total composition. In a preferred embodiment, both R^1 and R^2 are the hydrocarbon chain of eicosapentaenoic acid.

In a further embodiment, the anti-inflammatory compositions of the present invention further comprise DGDG. As used herein, "DGDG" is a digalactosyl-diacylglycerol as represented by the following structure II:



II

1,2-diacyl-(α-D-galactopyranosyl-(1'→6')-β-D-galactopyranosyl-(1'→3))-*sn*-glycerol

wherein R^1 and R^2 represent the hydrocarbon chain of a fatty acid containing from 10 to 25 carbon atoms and from 0 to 6 carbon-carbon double bonds. In addition, the anti-inflammatory compositions of the present invention may further comprise phosphatidyl choline ("PC"), as well as chlorophylls and carotenoids.

In yet another embodiment, a process of manufacturing an anti-inflammatory composition enriched with MGDG-EPA is disclosed. The process includes the steps of extracting marine algae with an extraction solvent, followed by phase separation with a first solvent protocol to yield an organic phase; fractionating the organic phase by polar chromatographic separation with a second solvent protocol to yield an MGDG-EPA enriched fraction; further fractionating the MGDG-EPA enriched fraction by non-polar chromatographic separation with a third solvent protocol to yield a further enriched MGDG-EPA; and removing solvents from the further enriched MGDG-EPA fraction to yield the anti-inflammatory composition.

In still a further embodiment, a method for treating inflammation comprising topically administering to an animal, including humans, an effective amount of a composition comprising a high content of MGDG-EPA is disclosed. The composition may be administered in various

forms, including emollients, ointments, capsules, tablets, drops, syrup, lozenges, suppositories, inclusions and aerosols.

Other aspects of the present invention will become evident upon reference to the attached figures and following detailed description.

Brief Description of the Drawings

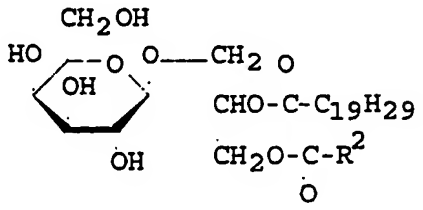
Figure 1 illustrates the components of a representative composition of this invention as resolved by thin layer chromatography (TLC).

Figure 2 presents the proton NMR spectra for MGDG-EPA.

Figure 3 compares the anti-inflammatory activity of a representative composition of the present invention (solid line) to that of a control (dashed line).

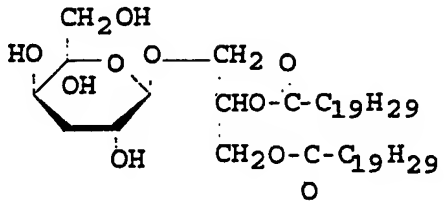
Detailed Description of the Invention

The present invention is directed to an anti-inflammatory composition containing a high content of MGDG-EPA. MGDG-EPA is identified above as structure I, wherein R^1 and R^2 represent a hydrocarbon chain of a fatty acid, and wherein at least R^1 or R^2 is the hydrocarbon chain of eicosapentaenoic acid ("EPA" - an omega-3 fatty acid containing 20 carbon atoms and 5 double bonds). For example, when R^1 of structure I is the hydrocarbon chain of eicosapentaenoic acid and R^2 represents the hydrocarbon chain of a non-EPA fatty acid, the corresponding MGDG-EPA has the following structure III:



III

Similarly, when both R^1 and R^2 of structure I is the hydrocarbon chain of EPA, the corresponding MGDG-EPA has the following structure IV:



IV

As used herein, the term "fatty acid" refers to a class of organic compounds containing a saturated or unsaturated, branched or unbranched, substituted or unsubstituted, hydrocarbon chain which terminates with a carboxyl group. The hydrocarbon chains of the fatty acids of the present invention preferably contain from 10 to 25 carbon atoms and from 0 to 6 carbon-carbon double bonds, and more preferably from 12 to 22 carbon atoms. Representative examples of fatty acid include (but are not limited to) lauric acid (12:0), myristic acid (14:0), palmetic acid (16:0), stearic acid (18:0), oleic acid (18:1) and eicosapentaenoic acid (20:5).

In a preferred embodiment of MGDG-EPA, both R^1 and R^2 of structure I above are the hydrocarbon chain of EPA. In a further preferred embodiment, the carbon-carbon double bonds of EPA are located at the Δ5,8,11,14, and 17 positions (i.e., *cis*-Δ-5,8,11,14,17-eicosapentaenoic acid).

As indicated above, the compositions of the present invention contain a high content of MGDG-EPA. Specifically, the composition may contain MGDG-EPA in an amount ranging from 35 to 95 percent by weight of the total composition, preferably from 40 to 80 percent by weight of the total composition, and most preferably from

50 to 70 percent by weight of the total composition. In addition to the values specifically identified above, all values falling within the above ranges are expressly incorporated herein.

The anti-inflammatory composition of the present invention may further include DGDG as identified above in structure II, wherein R^1 and R^2 represent the hydrocarbon chain of a fatty acid containing from 10 to 25 carbon atoms and from 0 to 6 carbon-carbon double bonds. DGDG may be present in the composition in an amount up to about 20 percent by weight of the total composition.

The anti-inflammatory composition of the present invention may further include phosphatidylcholine ("PC") in an amount up to 15 percent by weight of the total composition, as well as minor components of chlorophylls and/or carotenoids.

Eicosapentaenoic acid ("EPA") is present as a constituent, in an esterified form, of the monogalactosyl-diacylglycerol of the present invention. Although not intending to be limited to the following theory, the anti-inflammatory function of EPA is believed to involve the chemical messenger system responsible for stimulation of many of the physiological responses to inflammatory challenge, for example, reduced chemotaxis of inflammatory polymorphonuclear leukocytes. The chemical messenger system is part of the arachidonic acid ("AA") metabolic cascade, often referred to as the eicosanoid cascade. The cascade includes two enzyme systems which act upon AA to produce various prostaglandins, thromboxanes, leukotrienes and the like, which are important mediators of inflammation. Because of EPA's structural similarity to AA, EPA also is a substrate metabolized by the cascade. A structural difference between the two is the presence of an additional carbon-carbon double bond in EPA. Thus, the metabolites derived from EPA also retain the additional double bond not present in the metabolites of AA. As a

consequence, EPA metabolites greatly diminish, and in some instances eliminate, the physiological effectiveness of these metabolites relative to their AA counterparts. In addition, EPA is a competitive inhibitor of a key enzyme in the production of inflammatory prostaglandins and thromboxanes.

A primary step in inflammation is the release of AA from AA-phospholipid reservoirs found in cellular membranes. Exposure to EPA results in the replacement of AA and a concomitant increase of EPA-bearing phospholipid reservoirs. Upon inflammation challenge, the cascade commences with the release of AA or, if present, EPA by the enzyme phospholipase A_2 which is less reactive with EPA-phospholipids than AA-phospholipids. Inflammation occurs as a result of AA metabolism whereas the metabolism of EPA does not result in inflammation. Prophylactic EPA therapy to prevent inflammation is based upon the establishment of membrane phospholipid reservoirs enriched with EPA. The composition of the present invention provides a source of EPA as a substitute substrate in the eicosanoid cascade, thereby inhibiting or preventing inflammation.

The composition may be topically administered either prophylactically or in response to an inflammatory condition in an amount sufficient to prevent or diminish inflammation. Formulations for topical administration include tablets, capsules, lozenges, suppositories, syrups, drops, ointments, creams, lotions, gels, oils, emollients, inclusions and aerosols. Topical administration is effective for the prevention or treatment of localized inflammatory conditions. For example, the composition may be administered as an emollient for sunburn, athlete's foot, acne, pruritis, eczema, psoriasis, atopic dermatitis, rheumatoid arthritis, and skin cancer; as an aerosol for the treatment of bronchial inflammation and esophagitis; as an

enteric coated pill or tablet for inflammatory bowel disease or suppository for rectal inflammation; as a lozenge for esophagitis; and as drops for inflammation of the eye, ear or nose.

The composition of the present invention is applied at a concentration and frequency which provides effective prevention or treatment of inflammation. The formulation of the composition, as well as administered concentrations and schedules, can be determined by one skilled in the art, and will be depend on a number of factors, including the avenue of administration, location and severity of inflammation and composition formulation. In the practice of this invention, the composition containing a high content of MGDG-EPA is preferably applied at a formulation concentration ranging from 0.1 to 10 percent by weight of the formulation.

The efficacy of the anti-inflammatory compositions of this invention may be determined by appropriate assays. For example, a preferred general efficacy assay for topical anti-inflammatory compositions is the mouse ear assay. In the mouse ear assay, the effectiveness of a composition in preventing inflammation is determined by measuring the mouse ear swelling in response to inflammation challenge. In the test, mouse ears are first treated with the anti-inflammatory composition and then subjected to exposure to a pro-inflammatory composition. The determination of the efficacy of the anti-inflammatory composition of the present invention by the mouse ear assay is described in Example 3 below.

The anti-inflammatory compositions of the present invention may be prepared from algae by sequential extraction, polar chromatography, and non-polar chromatography as disclosed in greater detail below. The genera of algae that may be extracted include (but are not limited to) Chlorella, Chaetoceros, Cyclotella,

Ellipsoidon, Isochrysis, Nannochloris, Nannochloropsis, Nitzschia, Phyaeodactylum, Porphyra, Porphyridium, Skeletonema, Thalassiosira, Gigartina, Monochrysis and Monoraphidium. Preferred species within the above genera include Chlorella minutissima, Chaetoceros gracilis, Chaetoceros muelleri, Cyclotella cryptica, Isochrysis galbana, Nannochloropsis salina, Nitzschia dissipata, and Phyaeodactylum tricornutum

The marine algae, from which the anti-inflammatory compositions of the present invention are obtained, are first seed cultured in small vessels (such as 15 L carboys) and then pilot scale cultured in shallow tanks, bioreactors, or ponds. The culture medium may be salinated freshwater or preferably sea water. The medium is preferably supplemented with nutrients, including (but not limited to) ammonium, bicarbonate, phosphate, iron, nitrate and trace minerals. Protocols for the large scale propagation of algae are described in, e.g., A. Richmond, Handbook of Microalgal Mass Culture, CRC Press, Boca Raton, FL (1986)(incorporated herein by reference in its entirety). The algae may be harvested once the density of the algal culture is sufficiently high, preferably when the optical density of the algal culture at 690 nm is 1.0. Harvesting may be accomplished by coarse screening of the algae and then concentration of the resulting algal slurry. Concentration of the algae by removal of water is typically accomplished by centrifugation, sedimentation, evaporation, flocculation, ultrafiltration, flotation, or a combination of these techniques. The resulting dewatered algae are suitable for extraction.

The algae are then extracted with an extraction solvent(s) to yield an extraction solvent extract. The extraction solvent or solvents solubilize the MGDG-EPA component(s) present in the algae. Suitable extraction solvents include polar organic solvents such as alcohols, esters, ethers, ketones, and aldehydes, mixtures thereof,

and mixtures thereof containing water. Preferred extraction solvents include methanol, ethanol, propanol, isopropanol, acetone. In a preferred embodiment, the extraction solvent is 90% (i.e., 180 proof) aqueous ethanol. The algae may be extracted with an extraction solvent at ambient temperature or preferably near the boiling point of the extraction solvent. The extraction process includes contacting the algae with the extraction solvent for a period of time sufficient to effect solvent solubilization of the MGDG-EPA components present in the algae. For example, extraction with 90% (180 proof) aqueous ethanol at a temperature near its boiling point for 10 minutes is preferred. Upon cooling the mixture, the ethanolic extract containing MGDG-EPA is separated from the residual algal solids by filtration to yield the extraction solvent extract.

The extraction solvent extract is then fractionated by phase separation with a first solvent protocol comprising an organic water-immiscible solvent and water. The organic water-immiscible solvent selectively solubilizes the MGDG-EPA present in the extraction solvent extract while the water serves to remove highly polar components from the extraction solvent extract such as sugars, amino acids, and other water soluble components. Suitable organic water-immiscible solvents include non-polar organic solvents such as hydrocarbons, ethers, and chlorinated hydrocarbons among others. Preferred organic water-immiscible solvents include pentane, hexane, heptane, petroleum ethers, diethyl ether, dichloromethane, and chloroform among others. In a preferred embodiment, the organic water-immiscible solvent is hexane. After thorough agitation of the extraction solvent with the organic water-immiscible solvent and water, the aqueous phase is separated from the organic phase. Removal of the organic solvents from the organic phase yields a crude algal lipid extract

containing the MGDG-EPA components. In a preferred embodiment, the extraction solvent and first solvent protocol comprises 90% aqueous ethanol, hexane, and water in a ratio of 1:1:1.

The crude algal lipid extract containing MGDG-EPA is then fractionated by polar chromatography. A solution of the crude algal lipid extract in a suitable solvent, such as 10% ethanol in hexane, is applied to a polar chromatographic column. The solid phase of the polar chromatographic column may be alumina, deactivated alumina, silicic acid, Florisil®, DEAE cellulose, or other suitable polar solid phase. In a preferred embodiment, the polar solid phase is deactivated alumina (described in greater detail in Example 1 below). The crude algal lipid extract is fractionated by polar chromatography by elution with a second solvent protocol. The second solvent protocol may be a suitable fractionating solvent or mixtures of solvents including water and mixtures of polar and non-polar organic solvents in various proportions. Suitable polar organic solvents include alcohols, esters, ethers, ketones, and aldehydes among others. Preferred polar organic solvents include alcohols and esters such as methanol, ethanol, and ethyl acetate. Suitable non-polar solvents include hydrocarbons, petroleum ethers, and chlorinated hydrocarbons among others. Preferred non-polar organic solvents include pentane, hexane, diethyl ether, dichloromethane, and chloroform. In preferred embodiment, the polar organic solvent is ethanol and the non-polar organic solvent is hexane. In a preferred embodiment, the second solvent protocol comprises water, ethanol, and hexane. In a typical polar chromatography, the column is eluted with increasingly polar solvents or solvent mixtures. For example, a polar column may be sequentially eluted with 10% ethanol in hexane, 40% ethanol in hexane, 100% ethanol, and 20% water in ethanol. When the crude algal lipid extract is fractionated by

deactivated alumina as the solid phase and with the above elution pattern, the fraction eluted with 20% water in ethanol contains the MGDG-EPA enriched fraction.

The enriched MGDG-EPA fraction is then further fractionated by non-polar chromatography to yield a further enriched MGDG-EPA fraction. Specifically, the enriched fraction, in a suitable solvent such as ethanol/water (1:1), is applied to a non-polar chromatographic column. The solid phase of the chromatographic column may be any suitable non-polar or reverse phase solid phase. In a preferred embodiment, the non-polar solid phase is reverse phase silica, and in a most preferred embodiment, the non-polar solid phase is reverse phase octadecylsilyl silica (ODS). The further enrichment of the MGDG-EPA fraction is accomplished by elution with a third solvent protocol. The third solvent protocol may be any suitable fractionating solvent or mixtures of solvents including water and mixtures of polar organic solvents in various proportions. Suitable polar organic solvents include alcohols, esters, ethers, ketones, and aldehydes among others. Preferred polar organic solvents include alcohols and esters such as methanol, ethanol, and ethyl acetate. In a preferred embodiment, the polar organic solvent is ethanol and the third solvent protocol comprises water and ethanol in varying proportions. In a typical non-polar chromatography, the column is eluted with decreasingly polar solvents or solvent mixtures. For example, a non-polar column may be sequentially eluted with 50% (100 proof) aqueous ethanol, 70% (140 proof) aqueous ethanol, 80% (160 proof) aqueous ethanol, 90% (180 proof) aqueous ethanol, and 100% (200 proof) ethanol. When the enriched MGDG-EPA fraction obtained from polar chromatographic separation was further fractionated by non-polar chromatography with ODS as the solid phase and with the

above solvent system, the further enriched MGDG-EPA fraction eluted with 90% (180 proof) aqueous ethanol.

The following examples are offered by way of illustration, not limitation.

EXAMPLES

Example 1

This example illustrates the preparation of an anti-inflammatory composition of the present invention.

The marine microalga Chlorella minutissima was grown in cultivation tanks 4' x 10' with a paddle wheel system for agitation and a culture medium depth of 10"-12". The culture medium consisted of seawater, supplemented with ammonium nitrate (8 mM), sodium bicarbonate (5 mM), potassium dihydrogen phosphate (0.04 mM), and iron versenate (0.02 mM). The cultures were grown in full sunlight, or in plastic covered green houses, and the nutrient levels were measured weekly and additional nutrients added as required. When the cultures reached an optical density of 1.0 (measured at 690 nm) the algae were harvested. Fifty percent of each of 6 culture tanks was harvested giving a total of approximately 700 gallons.

The culture was pumped through a mixing tower where metered amounts of coagulant (Calgon WT-2511) were added with rapid agitation. The mixture next passed into a non-stirred column where metered amounts of flocculent (Betz Polymer 1160) were added, and thence into settling tanks. Settling was rapid and a large proportion of supernatant water was discarded. After further settling overnight, in excess of about 90% of the supernatant liquid was discarded, leaving a total volume of approximately 25 gallons. To this was added 3.64 kg of diatomaceous earth (DE) as a filter aid. Further rapid settling occurred, thus allowing the removal of more

supernatant water to leave 12 gallons of slurry. The slurry was vacuum filtered to yield a cake weighing 11.4 kg. The dry weight of algae contained in the cake was 686 gm.

The algal cake was extracted in 1.5 kg aliquots by adding to 4 L of boiling 90 percent aqueous ethanol, bringing the mixture back to a boil (5-10 minutes), and then continuing boiling for 10 minutes. The extract was obtained by filtration through a Buchner funnel under vacuum to remove solids. Water and hexane were added to the cooled extract to provide a final proportion (extract/hexane/water) of 1:1:1 and the mixture was agitated well and allowed to separate into two phases. The lower, aqueous ethanol phase contained water soluble materials, including sugars, amino acids, and some pigments. The upper hexane phase contained the crude lipid extract. The solvent was evaporated from the upper phase and the residue taken up in a minimal amount of 10% ethanol in hexane. At this stage small aliquots were taken for determination of dry weight, for thin-layer chromatography, and for fatty acid analysis by transesterification and gas chromatography. Total lipid extract was 98.8 gm, or 14.4% of dry weight of algae.

The crude lipid extract, dissolved in 10% ethanol in hexane to a total volume of 880 ml, was applied to a 4" x 24" alumina column prepared as follows: activated alumina (aluminum oxide) of a chromatographic grade (Aldrich Chemical Co., Brockman grade I, acidic, about 150 mesh) was deactivated by suspending it overnight in two volumes aqueous ethanol at a mixture of 70 parts ethyl alcohol to 30 parts water. The deactivated alumina was then packed into the column as a slurry in the water/alcohol mixture. The column was washed well with four volumes of absolute ethanol to remove the water, followed by a wash with one column volume of 40% ethanol in hexane and two column volumes of 10% ethanol in hexane.

The crude lipid extract was pumped onto the column and eluted with 4 L of 10% ethanol in hexane, 3 L of 40% ethanol in hexane, 4 L of 100% ethanol, 4 L of 20% water/80% ethanol and 3 L ethanol wash. All fractions were vacuum evaporated, the residues taken up in 10% ethanol in hexane and samples applied to TLC plates for analysis of lipid composition.

The 20% water/80% ethanol fraction, which contained the MGDG-EPA, was evaporated to dryness (weight - 12.4 gm) and taken up in ethanol/water 1:1. The MGDG enriched fraction was then further separated on a 2" x 12" reverse-phase column of ODS (Preparative C18, 125A, Millipore Corp.) which was prepared as follows: the ODS-silica was slurried with absolute ethanol and packed in the column in ethanol. After a wash with 1 L of absolute ethanol, the column was washed with 1 L of 75% ethanol/25% water, then 1.5 L of ethanol/water 1:1. The MGDG-EPA-containing fraction from the alumina column was divided in two, and one portion (~6.2 gm) at a time separated on the ODS column in two separate runs.

Elution was with 1 L of 50% aqueous ethanol, 1 L of 70% (140 proof) aqueous ethanol, 1.5 L of 80% (160 proof) aqueous ethanol, 1 L of 85% (170 proof) aqueous ethanol, 1.5 L of 90% (180 proof) aqueous ethanol and 2.5 L of absolute ethanol. In each run, the 90% aqueous ethanol fraction contained the bulk of the MGDG-EPA (approx. 90% of the total) as determined by TLC. These 90% fractions from the two runs were combined and evaporated (total weight 10.2 gm). The composition of the combined fractions was approximately 60% MGDG-EPA, 30% eicosapentaenoic acid believed to be bearing sulphoquinovosyldiacylglyceride ("SQDG-EPA"), 7% DGDG and 3% PC (with minor components of carotenoid and chlorophyll).

Example 2

This example illustrates the characterization of the MGDG-EPA components of Example 1.

A sample of the composition containing MGDG-EPA of Example 1 was subjected to preparative thin layer chromatography separation. Two major components were observed, MGDG-EPA and SQDG-EPA, as identified in Figure 1. MGDG-EPA was removed from the plate by scraping followed by elution from the silica. The proton NMR spectra of the MGDG-EPA component is presented in Figure 2. In addition, a portion of the MGDG-EPA was transesterified and the resulting fatty acid esters were analyzed by gas chromatography. The identity of the components of the fatty acid ester mixtures was made by comparison to retention time with known standards run immediately before and after the samples, and by carbon number plotting. The fatty acids of the MGDG-EPA component are (in percent by weight) presented in Table 1 below:

Table 1

Fatty Acid	Percent MGDG
12:0	tr
14:0	5.4
16:0	0.2
16:1	1.4
16:5	---
18:1	---
20:2	---
20:4	1.5
20:5	91.5
22:4	---

In Table 1 above, the first number of the fatty acid represents the number of carbon atoms in the fatty acid, and the number following the colon represents the number of double bonds in the hydrocarbon chain of the fatty acid. The identity of the major fatty acid constituent (i.e., 20:5) as eicosapentaenoic acid was confirmed by mass spectrometry. In the practice of this invention, EPA constitutes 50-99% of the fatty acids esterified to MGDG, preferably from 70-95%, and more preferably from 80-90%.

Example 3

This example illustrates the efficacy of a composition of the present invention as an anti-inflammatory agent in the generic mouse ear test.

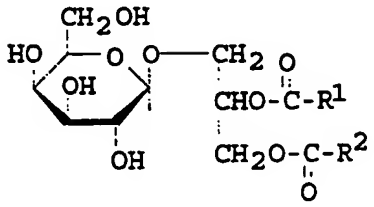
Both ears of mice were treated with either petrolatum (Vaseline®) or a formulation of petrolatum and a representative composition of this invention containing 85% by weight MGDG-EPA, 15% by weight DGDG, and trace chlorophylls (with EPA totaling 50% by weight of the total composition). The formulation used in this study contained 2% by weight of the above composition and 98% by weight petrolatum. Treatment involved the application of 5 mg of either petrolatum by itself or the above 2% formulation. Application was accomplished with a wooden stick to each ear at 9:00 a.m. and 5:00 p.m. for 5 consecutive days. Five mice (i.e., 10 ears) were used for both the control (i.e., petrolatum only treatment) and test animals (i.e., animals treated with the above 2% formulation). Two hours after the last application, 10 μl of 10% croton oil in acetone was applied to each ear to induce inflammation. The total thickness of the ears was measured with a micrometer having a measuring surface of 1 cm diameter, which is approximately the size of the ear. Measurements were taken immediately before challenge (t = 0) and 2, 4, 8, and 24 hours after croton oil

application. The results of this experiment are presented in Figure 3, with ordinate representing the change in ear thickness in microns (i.e., swelling) as determined from t = 0 to t = 2, 4, 8 and 24 hours. Figure 3 illustrates a statistically significant depression of ear swelling by the 2% formulation (solid line) at 4, 8, and 24 hours after induction of inflammation compared to the petrolatum control (dashed line).

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except by the appended claims.

Claims

1. An anti-inflammatory composition comprising a monogalactosyldiacylglycerol having the structure:



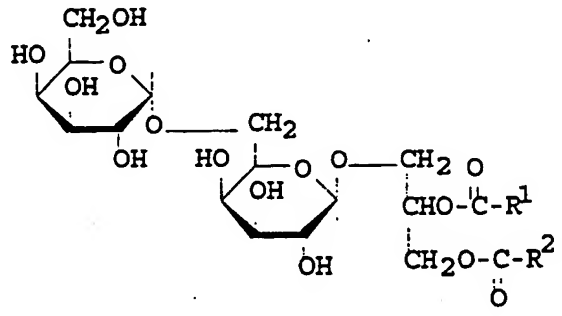
wherein R^1 and R^2 represent a hydrocarbon chain of a fatty acid containing from 10 to 25 carbon atoms and from 0 to 6 carbon-carbon double bonds, wherein at least R^1 or R^2 is the hydrocarbon chain of eicosapentaenoic acid, and wherein the monogalactosyl-diacylglycerol is present in the composition in an amount ranging from 35 to 95 percent by weight of the total composition.

2. The composition of claim 1 wherein both R^1 and R^2 are the hydrocarbon chain of eicosapentaenoic acid.

3. The composition of claim 1 wherein the monogalactosyldiacylglycerol is present in the composition in an amount ranging from 40 to 80 percent by weight of the total composition.

4. The composition of claim 1 wherein the monogalactosyldiacylglycerol is present in the composition in an amount ranging from 50 to 70 percent by weight of the total composition.

5. The composition of any one of claims 1-4 further comprising a digalactosyldiacylglycerol having the structure:



wherein R^1 and R^2 represent the hydrocarbon chain of a fatty acid containing from 10 to 25 carbon atoms and from 0 to 6 carbon-carbon double bonds.

6. The composition of any one of claims 1-5 further comprising phosphatidylcholine.

7. A process of manufacturing an anti-inflammatory composition an MGDG-EPA, comprising:

extracting algae with an extraction solvent to yield an extract;

phase separating the extract with a first solvent protocol to yield an organic phase;

fractionating the organic phase by polar chromatographic separation employing deactivated alumina with a second solvent protocol to yield an enriched diglyceride fraction;

fractionating the enriched diglyceride fraction by non-polar chromatographic separation with a third solvent protocol to yield a further enriched diglyceride fraction; and

removing solvents from the further enriched diglyceride fraction to yield the anti-inflammatory composition.

8. The process of claim 7 wherein the algae is selected from the genera Chlorella, Chaetoceros, Cyclotella,

Ellipsoidon, Isochrysis, Nannochloris, Nannochloropsis, Nitzschia, Phyaeodactylum, Porphyra, Porphyridium, Skeletonema, Thalassiosira, Gigartina, Monochrysis and Monoraphidium.

9. The process of claim 7 wherein the alga is Chlorella minutissima.

10. An anti-inflammatory composition made according to the process of claim 7.

11. A method for treating inflammatory disorders comprising topically administering to an animal an effective amount of a composition comprising MGDG-EPA, wherein MGDG-EPA is present in the composition in an amount ranging from 35 to 90 percent by weight of the total composition.

12. The method of claim 11 wherein the composition is administered in the form of an emollient, syrup, oil, tablet, capsule, lozenge, aerosol, drop, suppository, ointment, or inclusion.

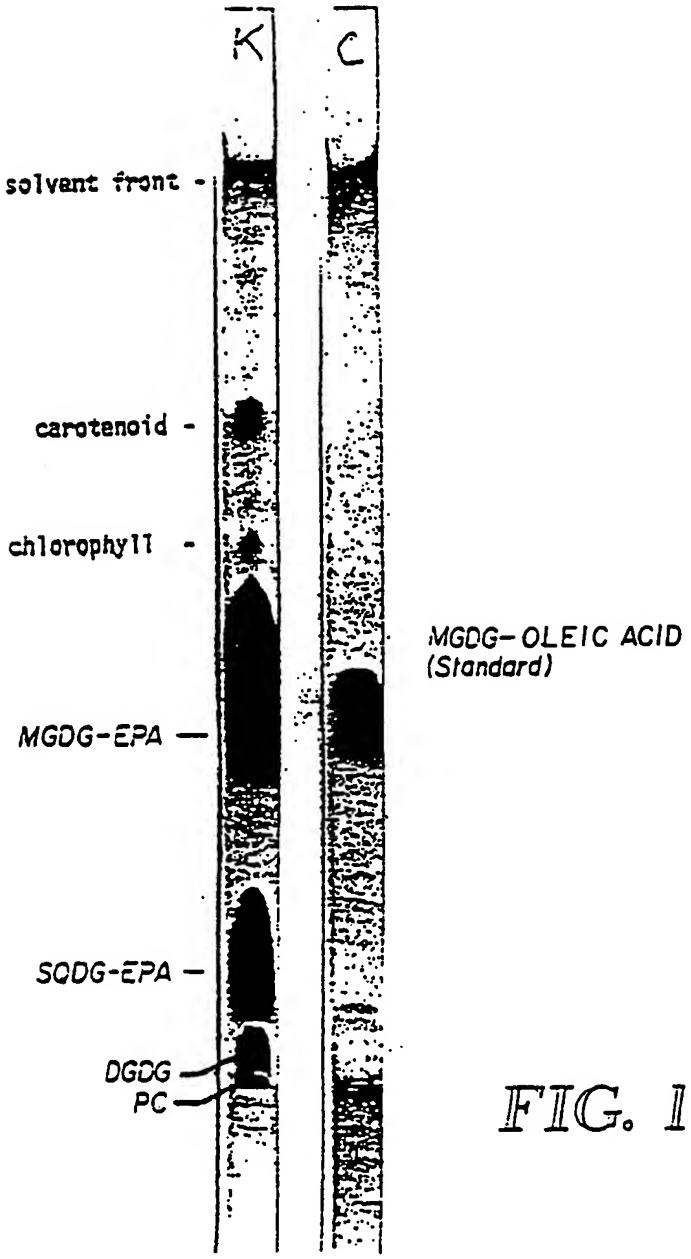
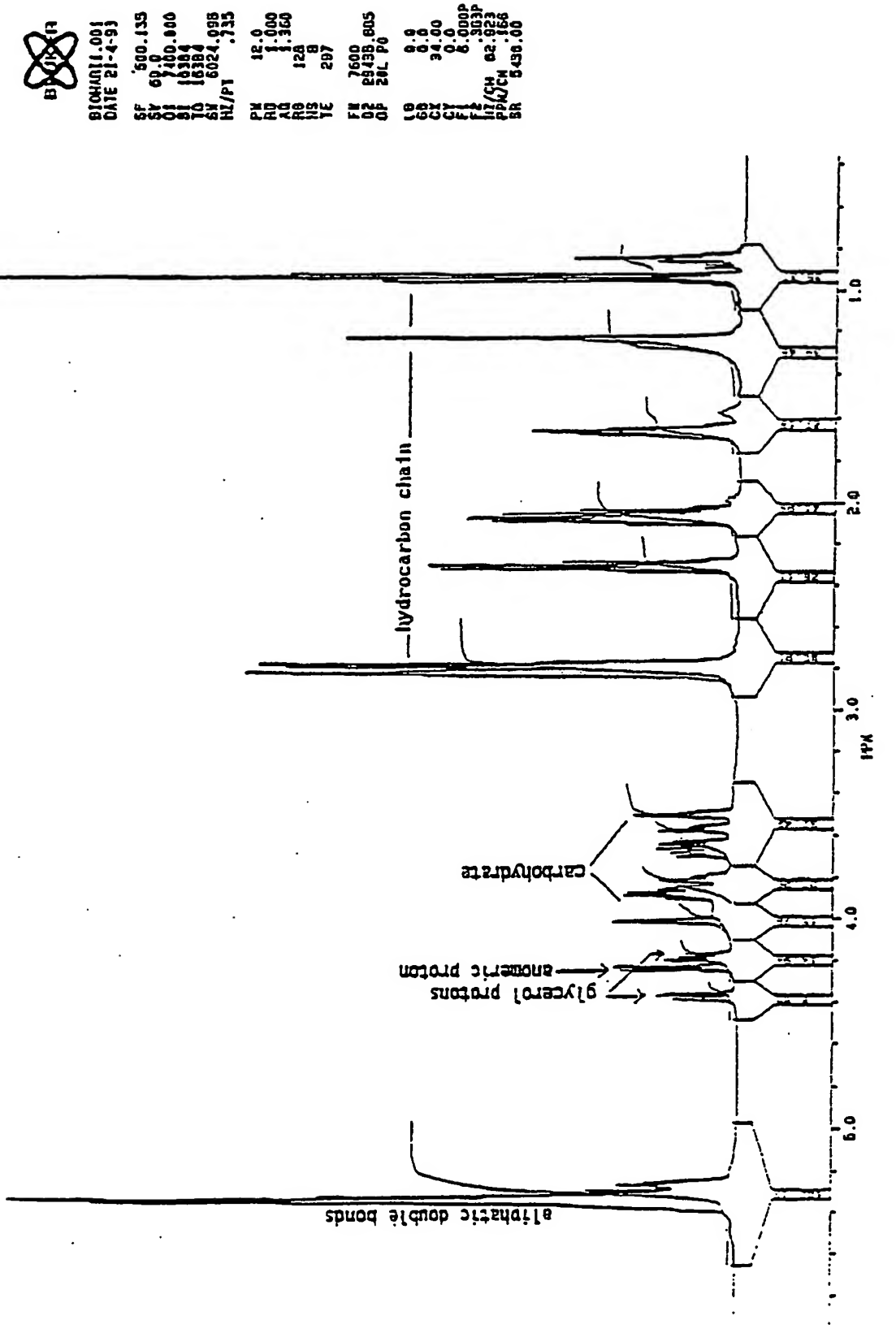

K
C
solvent front
carotenoid
chlorophyll
MGDG-EPA
SQDG-EPA
DGDG
PC
MGDG-OLEIC ACID
(Standard)

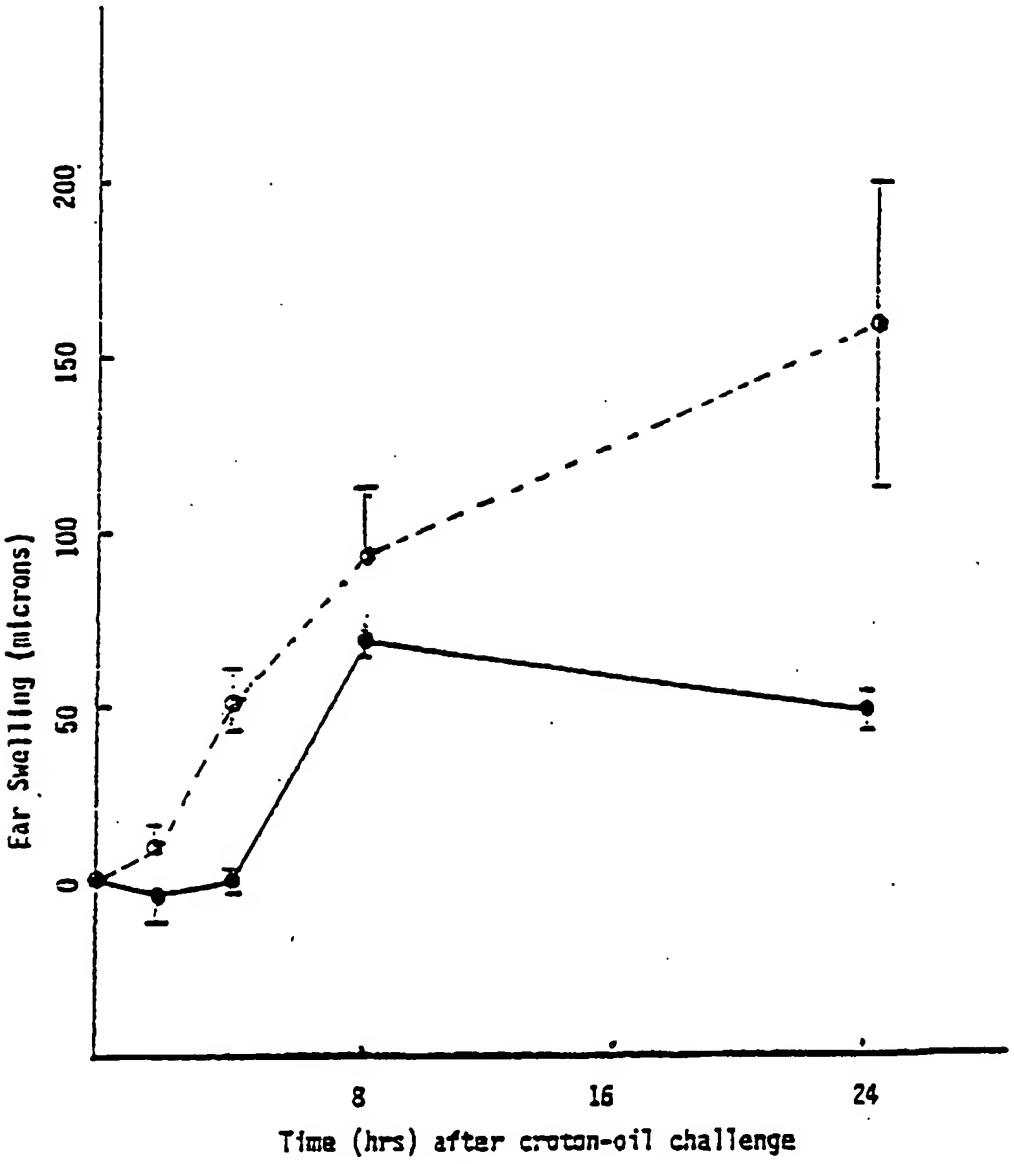
FIG. 1



MGDG-EPA

FIG. 2

FIG. 3


Ear Swelling (microns)
0
50
100
150
200
8
16
24
Time (hrs) after croton-oil challenge